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DIVALENT CATIONS AS CHARGE CARRIERS DURING TWO FUNCTIONALLY DIFFERENT MEMBRANE CURRENTS IN THE CILIATE *STYLONYCHIA*

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SUMMARY

We have investigated the ability of various divalent cations to carry current through membrane channels in ciliates activated by mechanical stimulation and by membrane depolarization. Both types of channels are identified as Ca^{2+} channels. Whereas Ba^{2+} ions and Sr^{2+} ions can replace Ca^{2+} ions as charge carriers during either kind of current, Mg^{2+} ions can carry the current evoked by mechanical stimulation but not the current elicited by membrane depolarization. Mn^{2+} ions did not carry either of these currents, and they reduced the currents carried by Ca^{2+} ions. Our experiments suggest that the mechano-sensitive and the voltage-senstive channels exhibit different selectivities for divalent cations.

INTRODUCTION

In ciliates, Ca^{2+} ions have been shown to be involved in the membrane depolarization following a mechanical stimulus and in the electrical excitation of the membrane. (Eckert, 1972; Machemer & de Peyer, 1977). In these two events, Ca^{2+} ions serve as charge carriers (during the membrane current flow: for ref. see Eckert & Brehm, 1979). It has therefore been suggested that the cell membrane of these protozoa contains two functionally different populations of Ca^{2+} channels: one being activated by a mechanical stimulus to the cell anterior, the other being activated by membrane depolarization. Recently, Ogura & Takahashi (1976) and Dulap (1977) found that the electrically excitable response in *Paramecium* disappeared after removal of the cilia, while the receptor responses to mechanical stimuli remained unaffected by deciliation (Ogura & Machemer, 1979). These results suggest that the voltage-sensitive Ca^{2+} channels are restricted to the ciliary membrane, whereas the mechanically activated Ca^{2+} channels reside in the membrane of the cell soma and/or of the ciliary base (which is unaffected by the deciliation). These findings lead to the question: do these two types of Ca^{2+} channels with different activation mechanisms and locations also differ in more intrinsic properties from each other. We have therefore compared the ionic selectivity of these channels to other divalent cations.

The hypotrich ciliate *Stylonychia* has three advantages as an experimental animal:

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(1) mechanical stimuli can be applied to surface areas of the membrane which are free of cilia; (2) the recording of receptor responses to mechanical stimulation is not hindered by trichocyst exocytosis; (3) the membrane currents recorded in voltage clamp are larger than in *Paramecium*. Our results show that the two types of Ca^{2+} channels can be clearly separated by their different permeability to Mg^{2+} ions as charge carriers.

Preliminary reports of parts of this work has been presented elsewhere (de Peyer, 1979; de Peyer & Deitmer, 1979).

METHODS

Cloned cells of *Stylonychia mytilus syngen I* were cultured in Pringsheim solution and fed with the phytomonad *Chlorogonium elongatum*.

For experimentation, the cells were washed and equilibrated in a solution of 1 mM-$CaCl_2$ + 1 mM-KCl buffered with 1 mM-Tris-HCl at pH 7·2–7·4, ('standard Ca^{2+} solution'). Ionic concentrations of the test solutions are listed in Table 1. In some experiments 0·5 mM-EGTA was added in order to ensure that the Ca activity was less than 10^{-8} M. The experiments were performed with the experimental chamber held at a temperature of 17–18 °C.

Electrical recording and mechanical stimulation have been described previously (de Peyer & Machemer 1977, 1978). The membrane potential was measured as the difference of an intracellular and an extracellular microelectrode, both filled with 1 M-KCl (resistance 30–60 MΩ). A third microelectrode, filled with 2 M-K-citrate, was inserted into the cell for current injection.

Membrane potential and membrane resistance were usually measured at the beginning of an experiment in the standard solution before introducing the test solution. The input resistance of the cell membrane was measured by injecting a small hyperpolarizing constant current pulse (1×10^{-10} A). Cells having a resistance of less than 50 MΩ in the standard solution were disregarded. Transfer to the test solution was done by perfusing the experimental chamber, exchanging at least 10 times the volume of the chamber of 1·5 ml.

The voltage clamp was performed by means of a conventional feedback system using a high gain differential amplifier (AD 171K). The membrane current was monitored by a current–voltage converter, connected to the bath.

Throughout the experiments, except for the Ba solution, the membrane resting potential was measured after transfer into the test solution. The holding potential

Table 1

Solution	Concentration (mM) Ca^{2+}	Mg^{2+}	Sr^{2+}	Ba^{2+}	Mn^{2+}	K^+	Cl^-	$Tris^+$
Standard								
Ca	1	—	—	—	—	1	4	1
Mg	—	1	—	—	—	1	4	1
Ca–Mg	1	1	—	—	—	1	6	1
Sr	—	—	1	—	—	1	4	1
Ba	—	—	—	1	—	1	4	1
Mn	—	—	—	—	1	1	4	1
Ca–Mn	1	—	—	—	1	1	6	1

in voltage clamp was always adapted to this resting potential if not stated otherwise. In the Ba solution, the change from 'standard solution' to 'test solution' was made under voltage-clamp conditions, to avoid an irreversible depolarization of the membrane.

RESULTS

1. *Mechanoreceptor current*

Fig. 1*A* shows membrane depolarizations elicited by mechanical stimuli given in 3 solutions in sequence: (1) standard Ca solution, (2) Mg solution, free of Ca, (3) standard Ca solution. The depolarization in the Ca solution consists of a mechanoreceptor potential and a graded action potential (de Peyer & Machemer, 1978). Although the two components cannot directly be identified from these depolarizations, they are here indicated by the two positive peaks in the time derivative (dV/dt) of the depolarization. In the Mg solution, the mechanical stimulus evoked a depolarization, which is characterized by a smaller amplitude, an exponential decay, and a single peak of maximum rate of rise. This depolarization is therefore assumed to be a pure mechanoreceptor potential. Its amplitude was 30 to 40 mV, and its maximum rate of rise was up to 5 V/s. The latter was about the same as the maximum rate of rise of the first component in the standard Ca solution. The depolarization in the Mg solution did *not* elicit the ciliary motor response which is typically seen in the Ca solution, following membrane depolarizations.

When Ca^{2+} was replaced by Mg^{2+}, the membrane resting potential changed little if at all, but the input resistance decreased considerably (Table 2). When the standard Ca solution was reintroduced, these changes reversed: a mechanical stimulus now elicited a depolarization consisting of receptor and action potential (Fig. 1*A*), which triggered the ciliary motor response.

Fig. 1*B* shows the membrane currents elicited by similar mechanical stimuli when the membrane was clamped to the resting potential, in order to eliminate the effect of voltage-dependent channels. Inward currents recorded under these conditions are therefore pure mechanoreceptor currents.

In the Mg solution the amplitude of the receptor current was often somewhat larger than in the Ca solution. The decay of this current had different time courses in the two solutions. The decaying phase of the receptor current in the Ca solution and in the Mg solution was plotted in a semi-log graph (Fig. 2). The receptor current in the Mg solution decayed with a single exponential time course with a time constant of 7·58 ms. In the Ca solution, the receptor current decay was approximated by two different exponential time courses with time constants of 1·8 ms and 7·22 ms. The slower of the two time courses in the Ca solution is similar to the single exponential time course in the Mg solution. The fast time course of the current decay in the Ca solution suggests that a Ca-specific relaxation mechanism may be involved.

The receptor *potentials* in the Ca solution had previously been shown as being largely dependent upon Ca ions (de Peyer & Machemer, 1978). Other ions in this standard Ca solution had been positively excluded from producing this inward receptor current. K^+ ions could be excluded as main charge carriers because the K^+ equilibrium potential was more *negative* than the resting potential (approximately

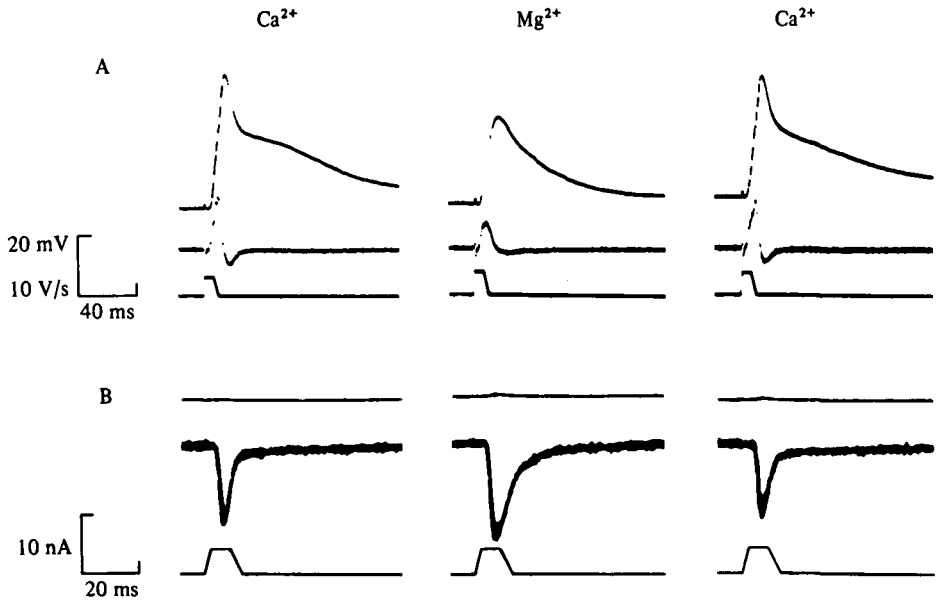

Fig. 1. (*A*) Intracellular recordings of membrane potential changes (upper trace) elicited by mechanical stimuli (lower trace) to the cell anterior, before and after replacement of Ca^{2+} by Mg^{2+}, and after returning to the standard Ca solution. The time derivatives of these potential changes, dV/dt, are shown in the middle trace. All records were obtained from the same cell. The upstrokes of action potentials were slightly retouched. (*B*) Membrane inward currents (middle trace) elicited by similar mechanical stimuli (lower trace) as in (*A*), when the membrane potential was clamped at its resting level (−50 mV, upper trace). All traces are three superposed records taken from the same cell.

−90 mV as compared to −50 mV). Changes in the Cl^- concentration of the bathing solution left both the depolarizing receptor potential and the inward receptor current unaltered. We have also eliminated $Tris^+$ as a possible ion carrying the receptor inward current; replacement of TrisCl by the impermeable organic ion K-HEPES (N-2-Hydroxyethylpiperazine-N′-2-ethanesulfonic acid) did not affect the receptor potential or the receptor current.

We have measured the reversal potential of the receptor inward current in the Ca and in the Mg solutions, and its dependence on the extracellular concentration of these ions. The membrane was clamped to increasingly positive levels, until the receptor current reversed (Fig. 3*A*).

The reversal potential, where mechanical stimulation did not evoke an increment in net current across the membrane, was then determined in 2–5 mV voltage steps (Fig. 3*B*). The mean reversal potential was measured to be $+13{\cdot}4 \pm 2{\cdot}6$ mV (mean ± S.D.) ($n = 10$) in the Ca solution, and $+3{\cdot}6 \pm 1{\cdot}8$ mV ($n = 10$) in the Mg solution.

Fig. 4 shows the results of a series of experiments, in which we have measured these reversal potentials in three more concentrations of Ca^{2+} and Mg^{2+} (0·25, 0·5 and 2·0 mM).

The reversal potentials of the receptor current were shifted to more positive values, when the concentration of Ca^{2+} and of Mg^{2+} was increased, and to more negative values when the concentration of Ca^{2+} and of Mg^{2+} was lowered. The slope of the

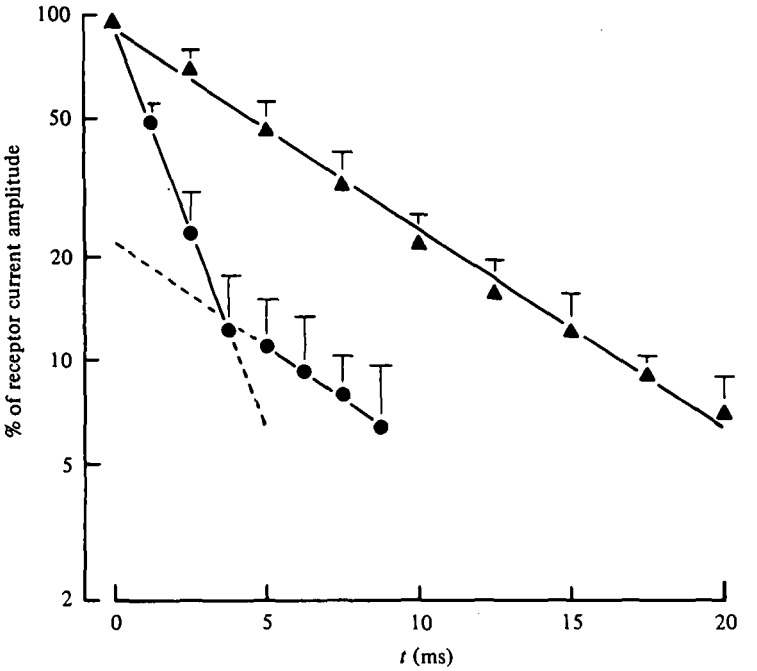

Fig. 2. Time courses of the receptor current decay in the Ca solution (circles) and in the Mg solution (triangles). The time constant of the first decaying phase in the Ca solution is 1·8 ms, and of the second decaying phase 7·22 ms. The time constant of the decay in the Mg solution is 7·58 ms. These time constants were determined by using the regression lines through the points. The vertical bars represent + one standard deviation.

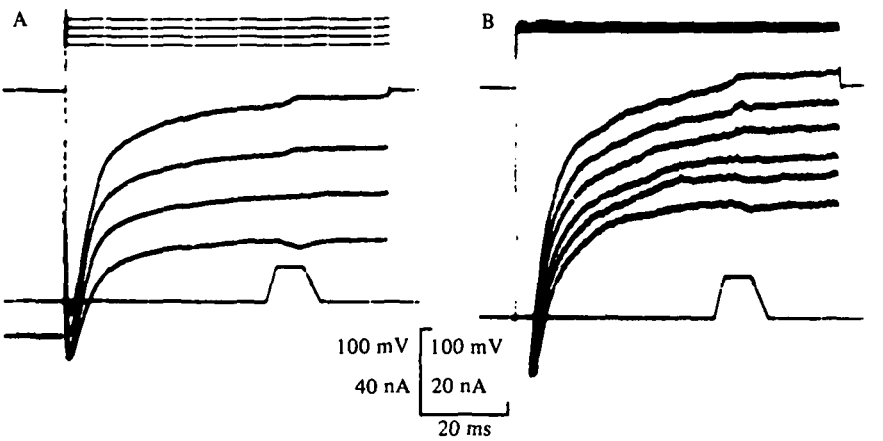

Fig. 3. Example of voltage-clamp recordings in the standard Ca solution to demonstrate how the reversal potential of the mechanoreceptor current was determined (holding potential −50 mV): (*A*) Depolarizing voltage steps of 10 mV difference (+50 to +80 mV from the resting potential) indicate the approximate reversal potential (here near to a voltage step of +60 mV). (*B*) Voltage steps of 2 mV difference (+60 to +70 mV from the resting potential) give the exact reversal potential of +14 mV.

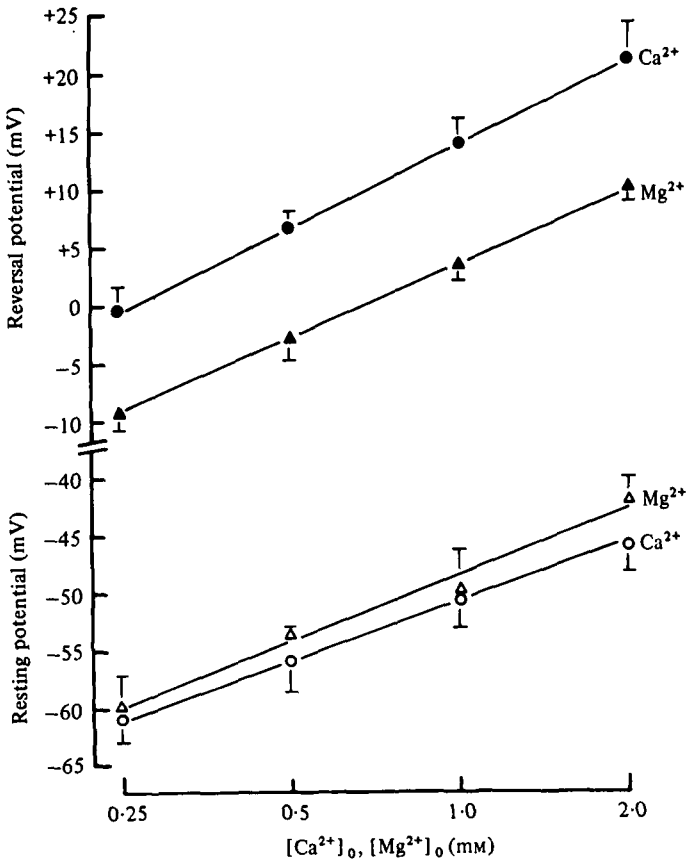

Fig. 4. Dependence of the reversal potential of the mechanoreceptor currents and the resting membrane potential on the extracellular concentration of Ca^{2+} and Mg^{2+}. The slopes of the reversal potential are 23·5 mV for Ca^{2+} (correlation coefficient r = 0·968) and 21·0 mV for Mg^{2+} (r = 0·971); those of the resting potential are 17·2 mV for Ca^{2+}, and 18·8 mV for Mg^{2+}. The bars represent + or − one standard deviation.

reversal potential change for a tenfold change in the concentration of Ca^{2+} and of Mg^{2+} was 23·5 mV and 21 mV, respectively. As can be seen in Fig. 4, the resting membrane potential also varied with the Ca^{2+} – and the Mg^{2+} – concentration. The slope was 17·2 mV for Ca^{2+} and 18·8 mV for Mg^{2+}.

We conclude from these experiments that both Ca^{2+} and Mg^{2+} ions can carry the mechanoreceptor current. However, neither the reversal potential (at least in Ca solution) nor its shift with changes in $[Ca]_o$ (or $[Mg]_o$) are in agreement with the value expected from the Nernst equation for an ionic channel perfectly selective to Ca^{2+} (or Mg^{2+}) ions (calculated E_{Ca} = 114 mV in the standard Ca solution, ideal slope 28·8 at 18 °C).

We have tested whether K^+ ions contribute to the mechanoreceptor current. Varying the extracellular K^+ concentration in both the standard Ca solution and in in Mg solution changed the reversal potential of the receptor current; increasing the K^+ concentration shifted the reversal potential to more positive values and vice versa. The slope of changes of the reversal potential of 5·3 mV in the Ca solution a

2 mV in the Mg solution indicates that the K^+ outward current activated by mechanical stimulation was relatively small. Its sign of polarity was opposite to that of the Ca^{2+} and Mg^{2+} inward receptor currents.

Applying the constant field theory (Goldman, 1943; Hodgkin & Katz, 1949) to the mechano-sensitive channel in the way Meves & Vogel (1973) did for the calcium voltage-dependent channel in squid axon, and Reuter & Scholz (1977) in cardiac muscle, we estimated a permeability ratio P_{Ca}/P_K for these channels. As the control experiments have shown that neither $Tris^+$ nor Cl^- ions could modify the receptor current, the only remaining charger carriers are Ca^{2+} and K^+ ions.

Then, the receptor membrane current (I_{rec}) is the sum of two currents:

$$I_{rec} = I_{Ca} + I_K \tag{1}$$

each of them being expressed by their constant field equation.

$$I_{Ca} = 4P_{Ca}\frac{(V-V')F^2}{RT}\cdot\frac{\alpha[Ca]_i\exp(2V'F/RT)-\alpha[Ca]_o\exp(-2(V-V')F/RT}{1-\exp(-2(V-V')F/RT} \tag{2}$$

$$I_K = P_K\frac{(V-V')F^2}{RT}\cdot\frac{\alpha[K]_i\exp(V'F/RT)-\alpha[K]_o\exp(-(V-V')F/RT}{1-\exp(-(V-V')F/RT} \tag{3}$$

where P_{Ca}, P_K, are Ca, K permeability; V, membrane potential; V', fixed charge potential resulting from the relative distribution of the fixed surface charges in the vicinity of the channels on both sides of the membrane, α, activity coefficient, being 0·975 for K^+ and 0·88 for Ca^{2+} at 1 mM concentration; [Ca], [K], concentration of these ions; F, R and T have their usual meanings; $\alpha[Ca]_i$ at about 10^{-7} M/l (Naitoh & Kaneko, 1937) can be neglected in the equation (2). At the reversal potential (V_R), $I_{rec} = 0$.

Re-combining (2) and (3) for V_R gives

$$V_R = \frac{RT}{F}\ln\frac{4P'_{Ca}\alpha[Ca]_o + P_K\alpha[K]_o}{P_K\alpha[K]_i} \tag{4}$$

with P'_{Ca} defined as

$$P'_{Ca} = P_{Ca}\left[\frac{1}{\exp((V_R-V')F/RT)+1}\right]. \tag{5}$$

With a $\alpha[K]_i$ in *Stylonychia* of 37 mM (de Peyer & Machemer, 1978), in the Ca standard solution, P'_{Ca}/P_K is then 1/0·056.

The real permeability ratio P_{Ca}/P_K will depend very much on the fixed charge potential V'. However, little is known about the amount of fixed charges in the vicinity of mechanoreceptor channels in protozoa. Nevertheless, a rather big negative surface potential was hypothesized in the vicinity of voltage-dependent channels (Eckert & Brehm, 1979). This means a high inside positive V'. Assuming V' to be about +80 mV (see also Reuter & Scholz (1977) for cardiac cells), the ratio P_{Ca}/P_K becomes 1/0·053. From this value, using equations (1), (2) and (3) we could calculate the relative contribution of Ca^{2+} and K^+ currents to I_{rec} at the resting potential. I_{Ca} (inward current) would be 94% and I_K (outward curent) 6% of the total I_{rec} (resting potential 50 mV).

A similar treatment could be applied to the Mg^{2+} permeability. However, in this

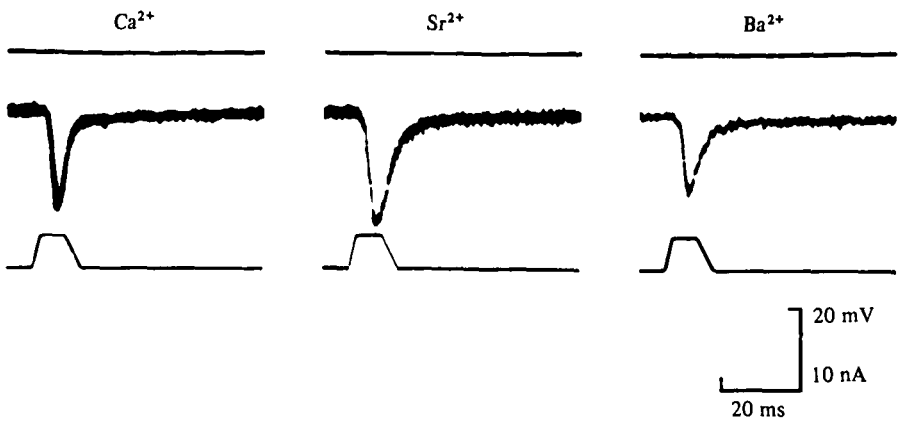

Fig. 5. Examples of inward currents (middle trace) recorded by voltage clamp (upper trace) and elicited by mechanical stimuli in the standard Ca solution, and in the test Sr and Ba solution.

case, the internal Mg activity which is assumed to be between 0·5 and 5·5 mM, Naitoh & Kaneko, 1973) cannot be neglected. Then, equation (4) becomes:

$$V_R = \frac{RT}{F} \ln \frac{4P'_{Mg}\,\alpha[Mg]_o + P_K\,\alpha[K]_o}{4P'_{Mg}\,\alpha[Mg]_i \exp((V_R - V')F/RT) + P_K\,\alpha[K]_i} \qquad (6)$$

with α is taken 0·89 for Mg^{2+} at 1 mM concentration.

Assuming no or little change in V' between Ca and Mg solutions, the ratio P_{Mg}/P_K will vary between 1/0·08 and 1/0·06, depending on $\alpha[Mg]_i$. Therefore, under the assumption made, there would be no or very little differences of selectivity of the mechanoreceptor channels to Ca and Mg.

Our observation that the mechanoreceptor channel is equally well permeable to Ca^{2+} and Mg^{2+}, leads to the question whether there is *any* ion selectivity of this channel. As stated before, $Tris^+$ ions and Cl^- ions could not carry current through this channel, K^+ ions little. Thus, the receptor channel appeared to be selective for divalent cations. To test this assumption we used various other divalent cations, such as Sr^{2+}, Ba^{2+} and Mn^{2+}, to see whether they could pass the mechanoreceptor channel.

Fig. 5 shows recordings of the receptor current from experiments in the Ca solution and in solutions where Ca^{2+} was replaced by Sr^{2+} and by Ba^{2+}. In both the Sr and Ba solutions, receptor currents were recorded. These divalent cations were the only possible charge carriers in the bathing solution; we therefore suggest that Sr^{2+} and Ba^{2+} are able to carry the receptor current. These receptor currents in the Sr and in the Ba solution reversed at positive membrane potentials as was already shown for the receptor current in the Ca and in the Mg solution. The reversal potential in the Sr solution varied between +10 mV and +15 mV (on average $+12{\cdot}3 \times 3{\cdot}1$ mV ($n = 6$)). The reversal potential of the receptor current in the Ba solution in two experiments was +10 mV and +8 mV.

When Ca^{2+} was replaced by Mn^{2+} in the bathing solution, the membrane resting potential increased from −50 mV to more than −75 mV, and the resting input resistance of the membrane decreased by more than one order of magnitude (see Table 2). In the Mn solution, mechanical stimuli did not elicit a depolarizing recep

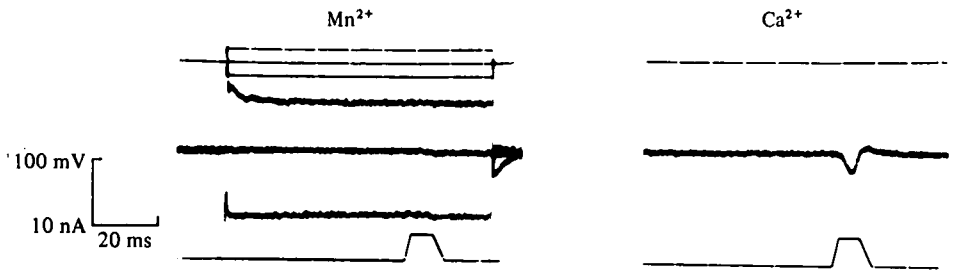

Fig. 6. Membrane currents recorded in the Mn (Ca-free) solution, and after returning to the Ca solution. No net receptor currents were recorded upon mechanical stimulation in the Mn solution, even when the membrane potential was shifted by ±20 mV from the holding potential (−75 mV). All records are from the same experiment.

Table 2

Solution	Resting potential (mV)	Resting input resistance (MΩ)	*n*
Ca	−50·6 ± 2·7	71·0 ± 6·0	9
Mg	−49·8 ± 1·5	14·4 ± 7·4	8
Ca–Mg	−45·0, −52·0	60, 60	2
Sr	−55·8 ± 2·9	30–80	10
Mn	−79·2 ± 4·1	1·5 ± 0·6	8
Ca–Mn	−41·8 ± 1·7	70·0 ± 14·1	4

Membrane resting potential and membrane resting input resistance in the 'standard Ca^{2+} solution', and in the test solutions containing different divalent cations. Figures are means ± one standard deviation of the mean; *n* gives the number of cells. In the Ca–Mg solution only two cells were tested, thus the individual figures are given here. For the resting input resistance in the Sr solution, a range of figures is given, because the input resistance was determined during the depolarization between the action potentials of the spontaneously firing cell.

potential nor an inward current during voltage clamp (Fig. 6*A*). This applies also to experiments where the membrane potential was displaced by voltage steps of ±20 mV. After the preparation had been returned to the standard Ca solution, a receptor inward current was recorded again, though much smaller than normal (Fig. 6*B*). Full reversibility of the mechanoreceptor properties was possibly hindered by partial, irreversible deterioration of the cell membrane due to exposure to the Ca^{2+}-free, Mn^{2+}-containing solution. We have repeated this type of experiment in the presence of 1 mM-tetra-ethyl-ammonium (TEA) in the Mn solution, in order to reduce the apparently large K^+ resting conductance of the membrane. Under these conditions, the membrane resting potential was approximately 10 mV less negative, and the input resistance greater than in the Mn solution. TEA (e.g. in the standard Ca solution) did not affect the receptor inward current by itself. In the Mn–TEA solution, mechanical stimulation did not evoke any changes in net current, confirming that Mn^{2+} ions do not pass the mechanoreceptor channel.

We have performed two other series of control experiments. First when Ca^{2+} was replaced by another divalent cation, we added 0·1 or 0·5 mM-EGTA to ensure that the Ca^{2+} activity did not exceed 10^{-7} or 10^{-8} M (In Ba and Mn solutions no EGTA was added because small amounts of EGTA in these solutions led to a quick death of the cells). Under these conditions neither the receptor potential nor the receptor

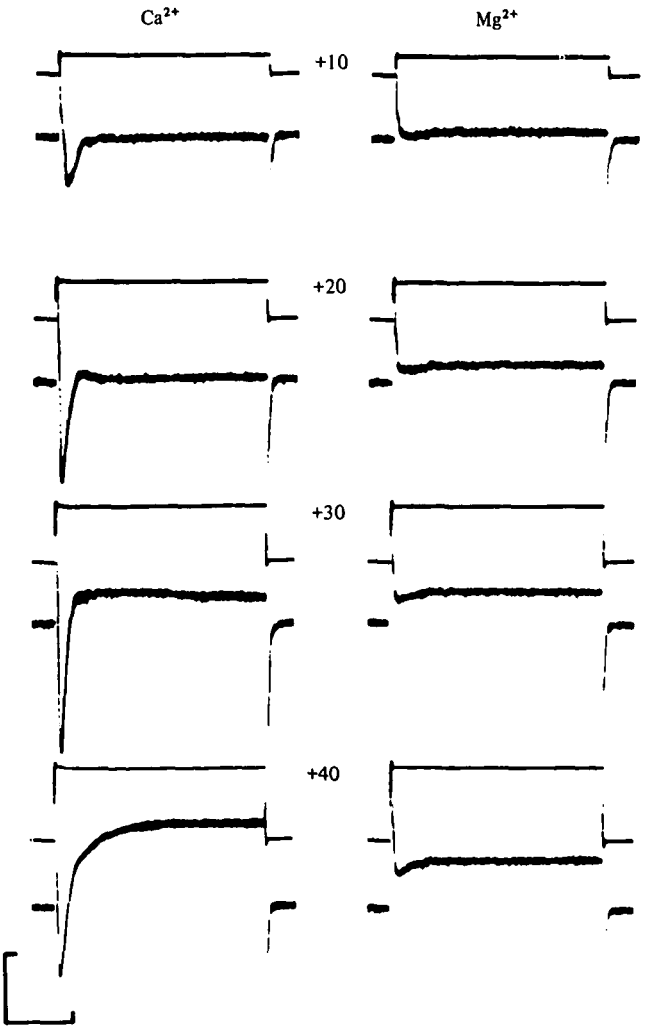

Fig. 7. Membrane currents (lower trace) recorded at four different depolarizing voltage steps of 60 ms duration (upper trace) in the standard Ca solution and in the Mg solution (Ca-free). The figures indicate the magnitude of the voltage steps. Note the absence of the early inward current in the Mg solution. The membrane was clamped at a holding level of −50 mV in the Ca solution, and of −49 mV in the Mg solution. All traces are three superposed records taken from the same cell. The vertical scale indicates 10 nA for all records except the bottom left, where it is 20 nA, the horizontal scale indicates 20 ms.

current in the Mg and in the Sr solution changed when compared to recordings in the same solutions without EGTA. Thus it was ascertained that the receptor potential and the receptor current measured were not dependent on residual amounts of Ca^{2+} present in these solutions.

In the second series of control experiments, we tested the ability of Mg^{2+} and of Mn^{2+} to cooperate or interfere with the receptor current in the presence of Ca^{2+}. In the Ca–Mg solution, the amplitude of the receptor inward current appeared to *increase* slightly compared to that in the standard Ca solution. In the Ca–Mn soluti

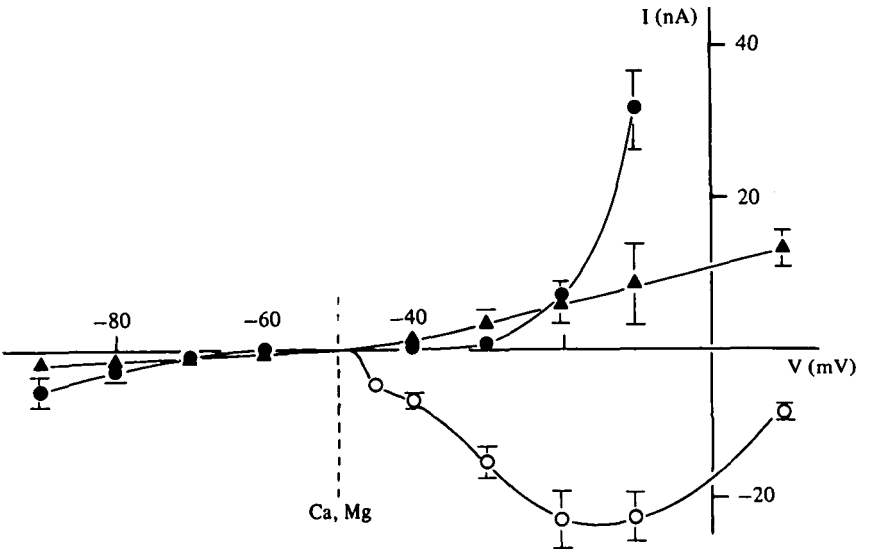

Fig. 8. Current–voltage relationship: early inward current (open circles) and steady-state current (filled circles) in the standard Ca solution, and steady-state current (triangles) in the Mg solution were plotted as functions of voltage steps from the resting level (−50 mV, dotted line). Each point in the hyperpolarizing direction is the mean from three, and in the depolarizing direction from six, experiments. The bars represent ± one standard deviation of the mean.

however, the mechanoreceptor current *decreased* by up to 50% as compared to that in the standard Ca solution.

II. *Voltage-sensitive inward currents*

Injection of depolarizing current into *Stylonychia* elicits graded action potentials which are largely dependent upon the extracellular Ca^{2+}-concentration (de Peyer & Machemer, 1977). Depolarizing voltage steps in voltage clamp evoked a fast inward current, carried by Ca^{2+}, and a delayed outward current, presumably carried by K^+.

Fig. 7 shows voltage-clamp recordings in the standard Ca solution, and after replacement of Ca^{2+} by Mg^{2+}. In the Ca solution, step voltages of up to −10 mV (equal to +40 mV steps) evoked early inward currents of increasing amplitude. These were followed by prominent delayed outward currents at voltage steps of $\geqslant +20$ mV. In the Mg solution, two major changes were observed: (1) no early inward currents were recorded upon depolarization and (2) the delayed outward current was greatly reduced.

Current–voltage relationships from 4 experiments in both the Ca and the Mg solution are plotted in Fig. 8.

The maximum Ca^{2+} inward current recorded was up to 25 nA at a membrane potential of −15 mV (equal to a depolarization of 35 mV from the resting level, see also Satow & Kung, 1979). At more positive membrane potentials, the peak inward current declined again. The delayed activation of the outward current began at depolarizations of around 20 mV and then greatly increased at more positive potentials. Hyperpolarizing voltage steps revealed inward-going rectification (of the steady-state current).

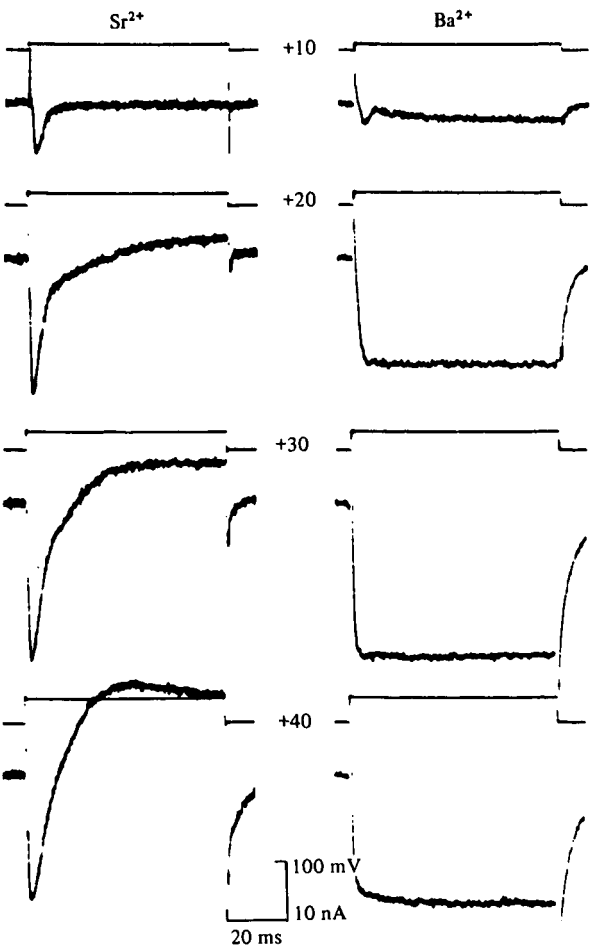

Fig. 9. Membrane currents (lower trace) recorded at four different depolarizing voltage steps (similar to those shown in Fig. 7). in the Sr and in the Ba solution. The figures indicate the magnitude of the voltage steps. The holding potentials were −55 mV in the Sr solution and −50 mV in the Ba solution. Note the persisting inward current in the Ba solution.

The steady-state *I–V* relationship in the Mg solution shows little outward rectification upon depolarization. The small outward-going rectification disappeared completely when 0·5 mM-EGTA was added to the Mg solution. This suggests that the outward rectification of the membrane might depend on the presence of a small but significant amount of extracellular Ca^{2+} as was also shown in *Paramecium* (Satow, 1978; Brehm, Dunlap & Eckert, 1978).

When Ca^{2+} in the bathing solution was replaced by either Sr^{2+} or Ba^{2+}, the membrane fired spontaneous all-or-none action potentials. These action potentials were much prolonged, up to 100 ms in the Sr solutions and several seconds in the Ba solutions, sometimes the membrane stayed depolarized for minutes (see also Naitoh & Eckert, 1968; de Peyer, 1973). In voltage clamp, large inward currents were recorded in the Sr and in the Ba solution during membrane depolarization. Fig. 9 shows typical recordings of membrane currents in these two solutions. Since no ot

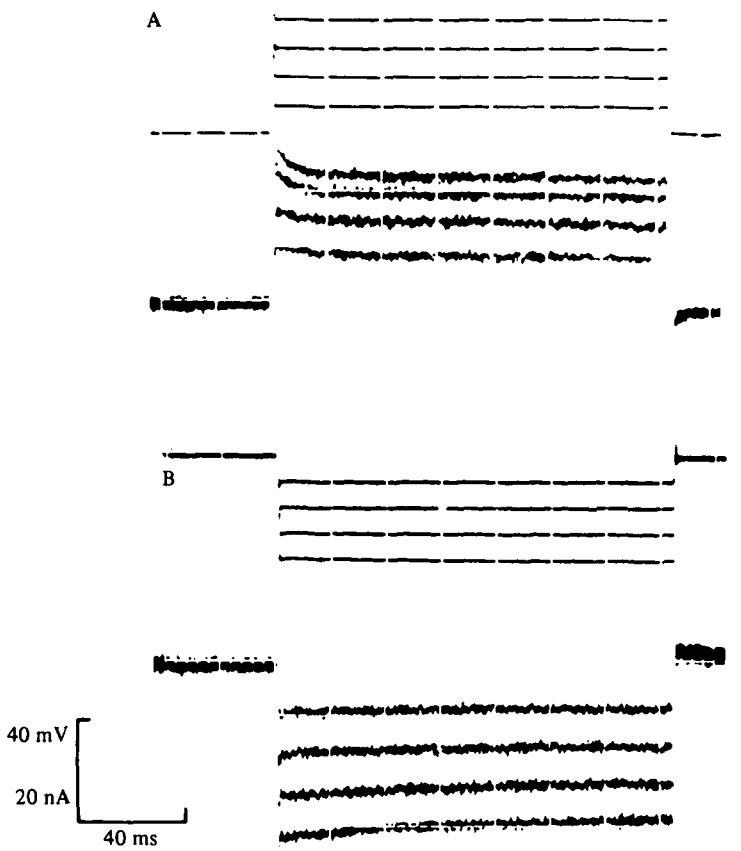

Fig. 10. Membrane currents (lower trace) in the Mn solution during voltage steps (upper trace) of up to +40 mV (*A*) and −40 mV (*B*) from the holding potential (−75 mV).

ions present in the bathing solution could carry an appreciable inward current (Tris did not carry any measurable amount of inward current), these inward currents were carried by Sr^{2+} and by Ba^{2+}. In the Sr solution the inward current decayed with a distinctly slower time course than the Ca solution, and the delayed outward current appeared to be activated later. At +20 mV and +30 mV voltage steps the Sr^{2+} inward current seemed to be composed of two components. More depolarizing voltage steps often revealed a transient outward current peak.

In the Ba solution there were large inward currents which maintained their full amplitude during the length of the voltage step and decreased very slowly when these steps were prolonged to several seconds. This is in accordance with the long duration of the Ba^{2+} action potential and suggest that there is little or no inactivation of the inward current and no activation of an outward current in the Ba solution.

When Ca^{2+} was replaced by Mn^{2+}, depolarizing voltage steps did not elicit any inward current (Fig. 10*A*). However, there appeared to be a substantial inward rectification, while hyperpolarizing voltage steps produced a linear current–voltage relationship (Fig. 10*B*).

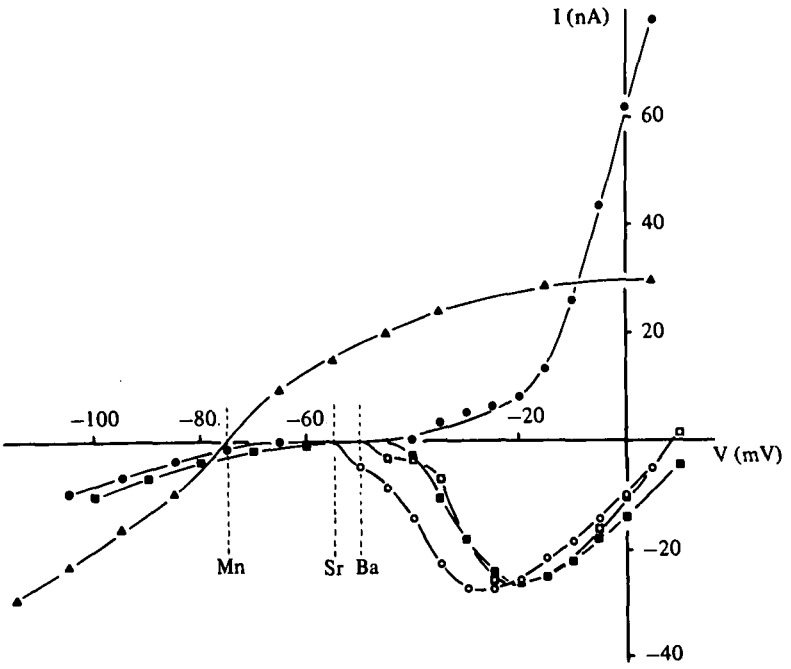

Fig. 11. Current–voltage relationships: *Early inward current* in the Sr solution (open circles), and in the Ba solution (open squares). *Steady-state current* in Sr (filled circles), in Ba (filled squares) and in Mn (filled triangles) solutions. The holding potentials in the various solutions are indicated by dashed lines. The inflections at the origin of the early inward currents in Sr and Ba solution were observed in all experiments.

Fig. 11. shows the current–voltage relationship in Sr, Ba and Mn solutions. The *I–V* relationship in the Sr solution resembled that obtained in the Ca solution. The maximum inward current was up to 28 nA, and the outward rectification in steady-state became prominent at depolarizations of 30 mV and more. In the Ba solution, both the early and the steady-state currents were in the inward direction. The peak of these inward currents was around 25 nA. In the Mn solution the steady-state *I–V* relationship revealed in the hyperpolarizing direction little, and in the depolarizing direction strong, inward-going rectification.

When Ca^{2+} *and* Mg^{2+} were present in the bathing solution, the fast inward current remained unaffected, indicating that Mg^{2+} did not significantly interfere with the Ca^{2+} influx through the voltage-sensitive channels. If, however, Mn^{2+} was added to the Ca solution (Ca–Mn solution) the early inward current was considerably reduced. Apparently Mn inhibits the early Ca inward current, presumably by competing with Ca for the same binding sites on the membrane (Hagiwara, 1975).

DISCUSSION

Our experiments have shown that the mechano-sensitive and the voltage-sensitive membrane channels in the ciliate *Stylonychia* differ in their permeability properties for divalent cations. While the voltage-sensitive channel behaves like a typical Ca channel, as has been described in many tissues (Hagiwara, 1975), the mechano-sensitiv

channel exhibits some quite different properties. A major difference is that Mg^{2+} can pass through the mechano-sensitive, but not through the voltage-sensitive, channel. The other divalent cations tested could either pass through both types of channels or none.

Mechano-sensitive current.

The inward current following a mechanical stimulus to the cell anterior appears to be a mixture of an inward current carried by divalent cations and an outward K^+ current. This has also been shown in *Paramecium* in the deciliated cell (Ogura & Machemer, 1979). The reversal potentials are expected to lie between the equilibrium potentials of the ions involved. The K^+-equilibrium potential in a solution containing 1 mM K approximates -90 mV in *Stylonychia* (de Peyer & Machemer, 1978). The equilibrium potential of Ca can be calculated at $+114$ mV, assuming an intracellular Ca activity of 10^{-7} M. The dependence of the reversal potential on the Ca^{2+} and the K^+ concentration indicates that the reversal potential should be much closer to the Ca equilibrium potential than measured in our experiments.

In this respect, the ratio $P_{Ca}/P_K = 1/0{\cdot}056$ could be a reasonable estimate in order to explain this discrepancy. However, this ratio does not hold any more for other Ca^{2+} concentations without additional assumptions. Calculating P_{Ca}/P_K for the other $\alpha[Ca]_o$ gives about a threefold increase of the ratio for a tenfold decrease of the extracellular Ca activity. Equation (5) shows that this effect may be due to either a change in the permeability itself or a change in V', or both. It is generally accepted that extracellular cations, especially divalent cations, can neutralize negative surface charges, shifting V' to a less positive value. Thereby, the maximum possible shift would be about 28 mV per tenfold increase in $\alpha[Ca^{2+}]_o$ (Hille, Woodhull & Shapiro, 1975). Under these conditions, if we tentatively let V' alone account for the change in the permeability ratio, we have to assume $V' \simeq 0$ mV or less in the standard Ca solution; every value of V' higher than that would give a shift bigger than the maximal expected 28 mV. This contradicts our previous assumption that V' should be high and positive, and although somewhat unlikely, we cannot exclude this possibility. Another complication may be that due to the large electromotive force for Ca^{2+} to flow into the cell, a transient local accumulation of Ca^{2+} at the inner receptor site may briefly abolish the originally high electrochemical potential. Furthermore, changes in the extracellular concentration of the given divalent cation may induce changes in their intracellular steady-state activities.

The decay of the receptor current depended upon the species of divalent cations carrying this current. In the Ca solution, the receptor current decayed with two exponential time courses. While the slow decaying phase of the Ca receptor current was similar to the single exponential decay of the receptor current carried by Mg, Sr, or Ba, the fast decaying phase was only present in Ca solutions. Our results suggest that a Ca-specific process may be involved in this fast decay, e.g. Ca-induced inactivation of this current, as has been postulated for the early voltage-dependent current in *Paramecium* (Brehm & Eckert, 1978), or the Ca-activated K^+ outward current as found in other cells (for ref. see Meech, 1978).

Voltage-sensitive current.

Ca^{2+}, Sr^{2+} and Ba^{2+} passed through the voltage-sensitive channel carrying the early inward current. Action potentials in the presence of these ions have been observed in ciliates (Naitoh & Eckert, 1968, 1969; Naitoh, Eckert & Friedman, 1972; de Peyer, 1973), as well as in many other Ca-dependent excitable tissues (Reuter, 1973; Hagiwara, 1975). Mn^{2+} ions, however, which have been reported to carry current in some excitable tissues (Ochi, 1971; Fukuda & Kawa, 1977), and Mg^{2+} ions did not carry charge through the ciliate membrane upon depolarization in our experiments. While the currents carried by Ca^{2+}, Sr^{2+}, and Ba^{2+} were similar in their maximum amplitude, they considerably differed in their time course. The Sr inward current was about twice as long in duration as the Ca inward current. This may be due to a different deactivation of membrane channels in the Ca and in the Sr solution, and/or to the delayed activation of the outward current. The Ba current, in contrast to the Ca and Sr currents, did not inactivate at all, as was recently shown also in *Paramecium* (Brehm & Eckert, 1978), and there was no activation of an outward current apparent in the Ba solution. The very slow decay of the Ba inward current during prolonged depolarizing voltage steps may indicate some intracellular accumulation of Ba^{2+}.

The steady-state I–V-curve also exhibited different characteristics in the presence of the various divalent cations tested. A large outward-going rectification of the membrane in Ca and in Sr solutions contrasts with an inward-going rectification in Mn, and a small rectification in the Mg solution. In Ba solutions, the steady-state I–V-relationship is similar to that of the peak inward current. It appears that the steady-state outward current and its voltage- and time-dependent characteristics greatly depend upon the species of divalent cations present. The outward-going rectification in Ca solutions may be due to a Ca-activated K current (Meech & Standen, 1975), which has also been postulated for *Paramecium* (Satow, 1978, Brehm *et al.* 1978). Our data suggest that this K current may also be turned on by Sr, but not by Ba. Ba even seems to suppress any K outward current although there is a continuous influx of Ba^{2+} into the cell during prolonged depolarization. In the Mg solution there was neither an inward current nor a substantial time-dependent outward current. This may also indicate that in *Stylonychia* the delayed activation of the voltage-dependent outward current is due to a calcium-specific process.

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